

Method based on S. J. Kim et al., *Biochem. Biophys. Res. Commun.* 1995, 212, 822-826.
 assay for SO_2 peroxide scavenging

Reagents: Tris-maleic acid buffer 55.6 mM Tris - 55.6 mM, pH 8.2 - R.H. made = TCB solution

40 mM Pyrogallol in 10 mM HCl. 10 mL dH_2O + 8.6 μL 11.6 N HCl + 50.5 mg pyrogallol (m.w. 126)

* Tried 2 mM pyr. First \rightarrow no absorbance Δ .

Reaction: 1. 8 mL TCB solution
 . 2 mL Pyrogallol solution
 . 1 mL SOD (10^4 U/mL) or 0.1 mL H_2O
 2.1 mL

Vertex, pipet rapidly into cuvettes.
 Kinetic protocol @ 420 nm, RT, blanked again H_2O . SOD/TCB blank = H_2O absorbance at 420 nm.

Plan: 2 mL reaction + C_3 dose-response. Lot 99L87R
 0 μM , 10 μM , 30 μM , 100 μM , 300 μM , 500 μM

Add as 0.1 mL solutions.

Ctrl = 0.1 mL TCB
 10 μM = $x(25 \text{ mM} \times 2.1 \text{ mL} \times 10 \mu\text{M})$ $x = 0.84 \mu\text{L} + 99 \mu\text{L TCB}$
 30 μM $x = 2.5 \mu\text{L} + 97 \mu\text{L TCB}$
 100 μM $x = 8.4 \mu\text{L} + 92 \mu\text{L TCB}$
 300 μM $x = 25 \mu\text{L} + 75 \mu\text{L TCB}$
 500 μM $x = 42 \mu\text{L} + 58 \mu\text{L TCB}$

500 μM \rightarrow absorbance off-scale

Same \downarrow SOD. Try 20 mM Pyr. and 10 mM Pyr. \bar{c}
 Same C_3 conc.

10 mM pyr. \rightarrow 2 mL 40 mM Py + 6 mL 10 mM HCl

* need to run + SOD control again with 10 mM Pyr.

EXHIBIT

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